Aerosol Stability of Bovine Parainfluenza Type 3 Virus

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ABSTRACT

Aerosols of bovine parainfluenza type 3 virus were generated with a Devilbiss 40 nebulizer from Eagle's minimum essential medium and nasal secretion from a noninfected calf and stored in a rotating drum at temperatures of 6°C or 32°C and relative humidities of 30% or 90%. The aerosols were sampled at seven minutes, one, two and three hours after the start of generation with an all glass impinger (AGI-30) and titrated for infectivity in cell cultures. Physical decay was determined by a rhodamine tracer technique. Media, temperature or relative humidity had little effect on the survival of parainfluenza type 3 virus during spraying (zero to seven minutes). During aging of aerosols at 32°C and 30% relative humidity, parainfluenza type 3 virus was less stable in Eagle's minimum essential medium than in nasal secretion from a noninfected calf, but at 6°C and 30% relative humidity, the virus was more stable in Eagle's minimum essential medium. At 32°C, the virus was less stable during aging at 90% relative humidity than at 30% relative humidity. The virus was consistently more stable during aging of aerosols at 6°C than at 32°C.

RÉSUMÉ

Cette expérience visait à produire des aérosols contenant le virus para-influenza 3 bovin, avec un pulvérisateur Devilbiss 40, à partir du milieu essentiel minimum d'Eagle et des sécrétions nasales d'un veau témoin; on entreposa ensuite ces aérosols, dans un cylindre rotatif, à la température de 6° ou 32°C, combinée à une humidité relative de 30% ou de 90%. On préleva des échantillons de ces aérosols, avec un appareil en verre (AGI-30), aux intervalles suivants: sept minutes, une, deux et trois heures après la pulvérisation; on titra ensuite leur infectivité, à l'aide de cultures cellulaires. On en détermina la dénaturation physique, à l'aide d'une technique de marquage à la rhodamine B. Les milieux, la température et l'humidité relative n'exercèrent que peu d'influence sur la survie du virus au cours de la pulvérisation, qui s'étala sur une période de sept minutes. Au cours du vieillissement des aérosols maintenus à 32°C, dans une humidité relative de 30%, le virus se révéla moins stable dans le milieu d'Eagle que dans les sécrétions nasales du veau témoin, contrairement à ce qui se produisit à la température de 6°C, dans une humidité relative de 30%. Au cours du vieillissement des aérosols maintenus à 32°C, le virus se révéla moins stable à l'humidité relative de 90% qu'à celle de 30%. Au cours du vieillissement des aérosols, le virus se révéla constamment plus stable à 6°C qu'à 32°C.

INTRODUCTION

Parainfluenza type 3 (PI-3) virus was first isolated from cattle with respiratory disease (16). Subsequent studies have indicated that the virus is widespread in the cattle population and have implicated the virus in the etiology of the shipping fever complex (15) and pneumoenteritis (11). This virus is believed to be transmitted by the airborne route, but no data are available in the literature on the stability of PI-3 virus in aerosols of nasal secretion, although Donaldson and Ferris (17) studied the effect of relative humidity (RH) on the viability of aerosols of PI-3 virus generated from Eagle's medium and stored at room temperature for periods of one second and five minutes. The present study was designed to determine the stability of PI-3 virus in aerosols of nasal secretion from a noninfected calf (NIBS)

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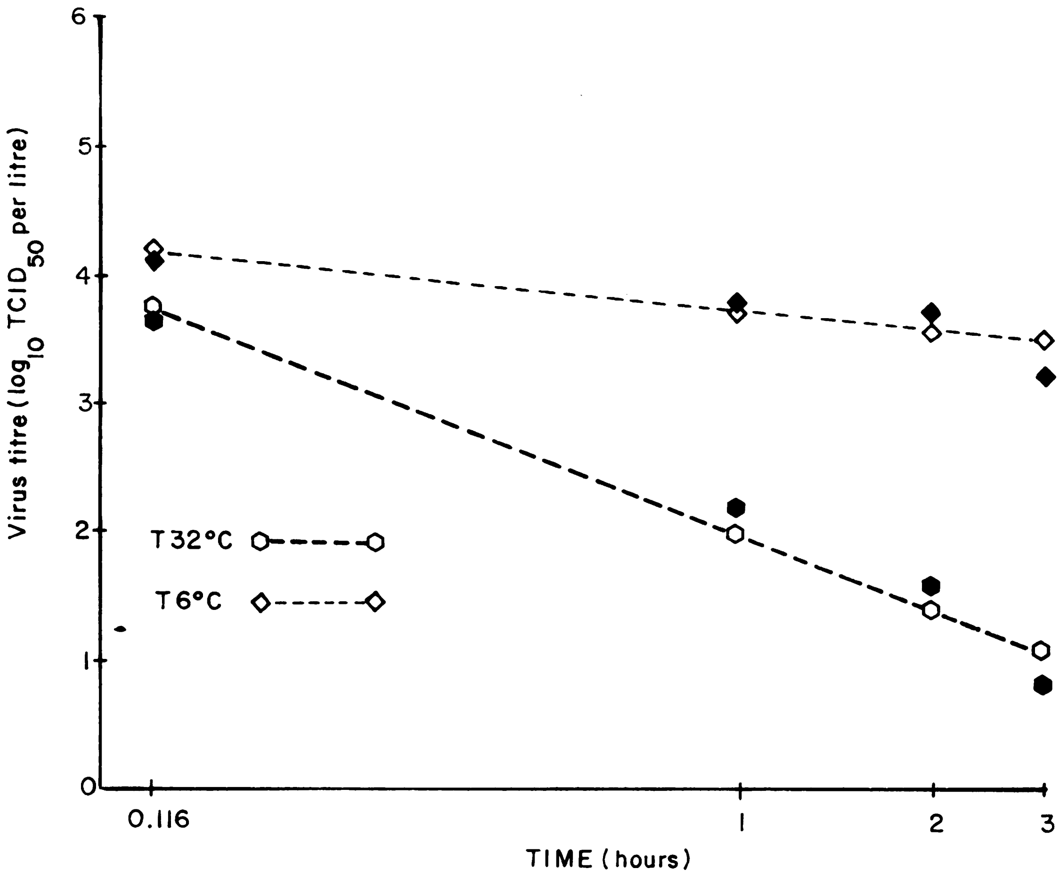

Fig. 1a. Effect of temperature (T) on biological decay of bovine PI-3 virus in aerosol of Eagle's minimum essential medium at 30% RH during three hour aging. ◇○ = average of estimated virus titre. ◆⬢ =average of observed virus titre.

and Eagle's minimum essential medium (EMEM) at temperatures of 6°C and 32°C and relative humidities of 30% and 90%.

We have previously reported aerobiological studies with infectious bovine rhinotracheitis virus (8) and similar studies with bovine adenovirus type 3 are described separately for the sake of greater clarity in a subsequent paper (9).

MATERIALS AND METHODS

Viral Cultivation

A local strain of PI-3 virus of bovine origin was supplied by Dr. M. Savan, University of Guelph. The virus had been passed several times in cell cultures and a stock produced in embryonic bovine lung (EBL) cell cultures had a titre of 10^{8} median tissue culture infectious doses ($TCID_{50}$) per ml. This stock virus was concentrated to $10^{9.9}$ $TCID_{50}$/ml by ultrafiltration (20), using a XM 100 A filter[1].

Collection of Nasal Secretion

Nasal secretion from a colostrum deprived calf which had been reared in isolation and lacked antibodies against PI-3 virus was collected as described by Rouse and Angulo (17) and stored at –70°C.

Aerosol Procedure

The concentrated stock of PI-3 virus was diluted 10^{-2} in NIBS or EMEM and stored

[1]Amicon Corporation, Lexington, Massachussetts.

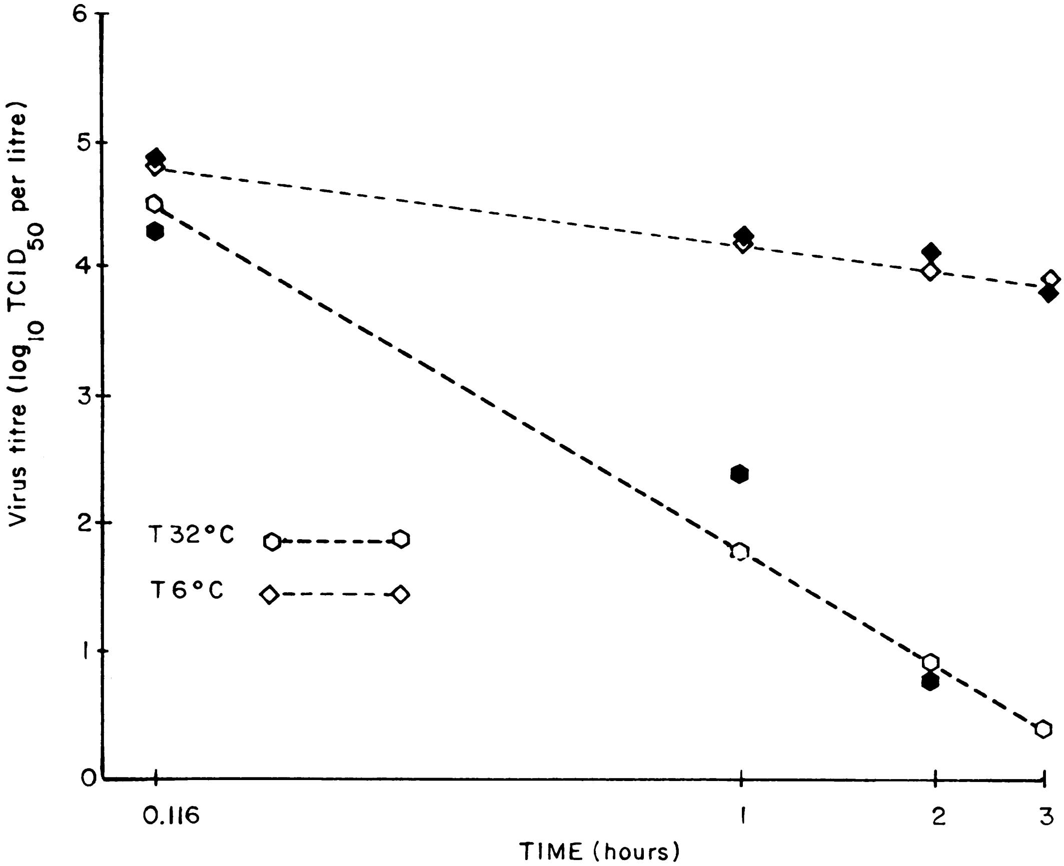

Fig. 1b. Effect of temperature (T) on biological decay of bovine PI-3 virus in aerosol of Eagle's minimum essential medium at 90% RH during three hour aging. ◇⬡ = average of estimated virus titre. ◆⬢ = average of observed virus titre.

in 3 ml vials in liquid nitrogen. Aerosols were generated from each of these viral suspensions with a Devilbiss 40 nebulizer[2] into a 200-litre stainless steel rotating (3 rpm) drum (18) for five minutes, followed by a stabilization period of two minutes. The detailed aerobiological procedures, including the operating pressure of the nebulizer, the determination of particle size of aerosols at seven minutes after generation, the sampling protocol and the determination of the physical decay at seven minutes after start of aerosol generation and one, two and three hours postgeneration were identical to those described previously (8). Aerosols were generated and stored at 32°C and 6°C and RH of 30% and 90%, and three replicates of each experiment were carried out. Based on a preliminary trial, 20 ml of EMEM were used as the collecting medium and the virus titrations were done immediately after aerosol collection with an AGI-30 (20) at seven minutes and one, two and three hours postgeneration.

Viral Assays

Viral concentrations in the nebulizer fluids and in the impinger fluids collected at seven minutes, one, two and three hours postgeneration were assayed on 70% confluent sheets of Madin-Darby bovine kidney (MDBK) cells in Linbro microtitre plates. The growth medium for the cells was minimum essential medium with Hank's salts[3] with 10% fetal calf serum (FCS) and

[2]Devilbiss (Canada) Ltd., Barrie, Ontario.

[3]Gibco, Grand Island, New York.

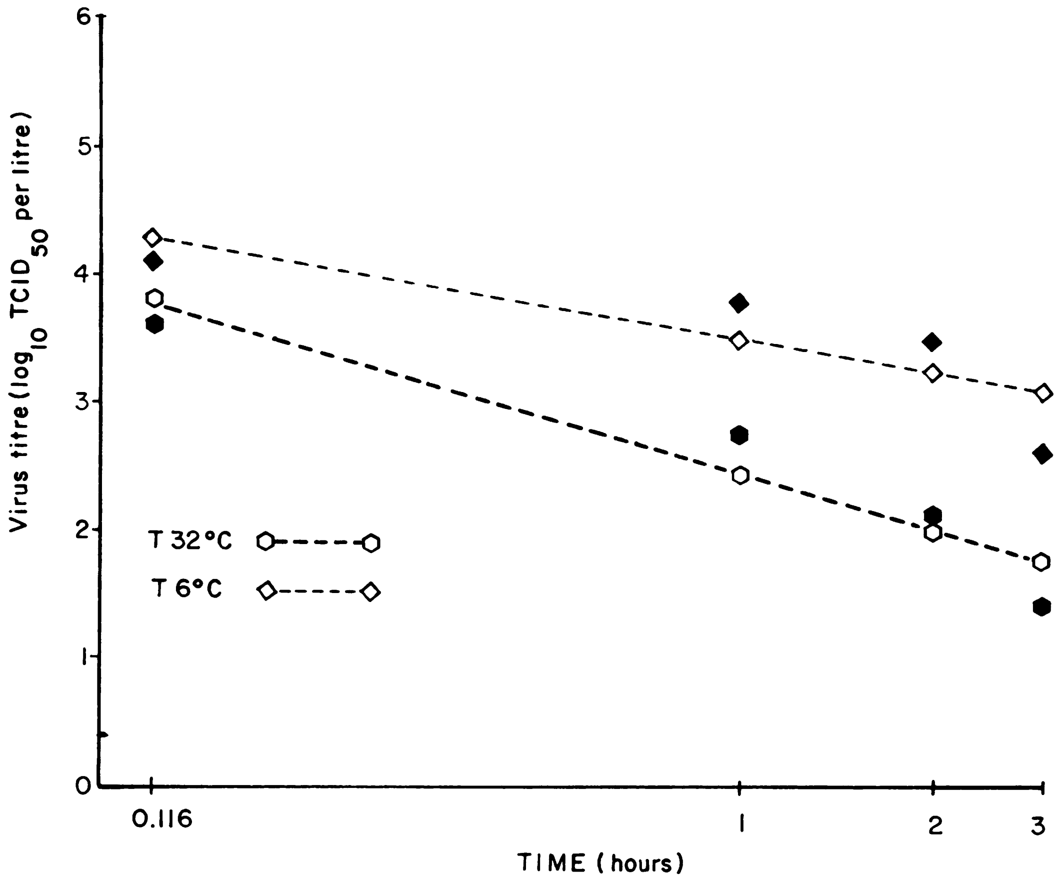

Fig. 1c. Effect of temperature (T) on biological decay of bovine PI-3 virus in aerosol of nasal secretion from noninfected calf at 30% RH during three hour storage. ◇○ = average of estimated virus titre. ◆⬢ = average of observed virus titre.

antibiotics (250 iu penicillin and 100 µg streptomycin per ml) and the maintenance medium was EMEM + 5% FCS with antibiotics, buffered with HEPES at a concentration of 0.045 M (10). Serial tenfold dilutions of each sample were prepared in maintenance medium and each dilution was inoculated into eight wells of MDBK cells. After incubation for six days, a hemadsorption test with 0.3% bovine red blood cells (2) was conducted on each well. Infectivity titres were calculated by the Spearman-Kaerber method.

Calculation and Statistical Analysis of Biological Decay Rates

The decrease in virus concentration due to loss of viral infectivity in the air (biological decay) was expressed as the biological decay rate. The biological decay rate during the spray period (seven minutes) was computed according to the formula $\frac{\log No - \log Nt}{t}$, in which log No represents the virus concentration at time O calculated from the volume and titre of the suspension which was nebulized, log Nt is the concentration of virus recovered at seven minutes postgeneration and t is time in hours (0.166 h). The biological decay rate (regression coefficient) during the storage period (seven minutes to three hours) was calculated by the method of least squares (19). Virus decay rates in aerosols of EMEM and BNS during spray and storage were subjected to the analysis of variance (19) to compare the effect on virus decay rate of medium at each temperature and humidity combination, of temperature within the same medium and RH and of RH within the same medium and temperature. A significance level of 1% was used.

When the results of the above comparison were significantly different during storage the virus inactivation curves (regression lines) for each comparison were plotted. The regression equation (19) used to calculate these virus inactivation curves was $y = a + b \log x$ where y is the expected average virus concentration (in $\log_{10}$ $TCID_{50}$/litre), a is the point where the line crosses the y-axis (y-intercept), b is the average decay rate (regression coefficient or slope of the line) based on three replicates and log x is the logarithm to base 10 of the time expressed in hours, commencing at seven minutes postspraying.

RESULTS

The concentration of virus aerosolized and recovered at seven minutes, one, two and three hours postgeneration from aerosols of BNS or EMEM at different temperatures (32°C, 6°C) and RH (30%, 90%) are recorded in Table I. During the initial seven minutes (spraying period) media, humidity and temperature had no significant influence on the virus decay rate, except in aerosols of EMEM, at 6°C when 30% RH was significantly more detrimental to the virus than 90% RH.

Regression lines for the significant effects of temperature, RH and medium on decay rates between seven minutes and three hours (aging period) are shown in Figs. 1-3. During aging, low temperature at both levels of humidity and in aerosols of both media was accompanied by the lowest biological decay rate (Fig. 1a, b, c, d). High RH was associated with the highest virus decay rate at 32°C (Fig. 2a,

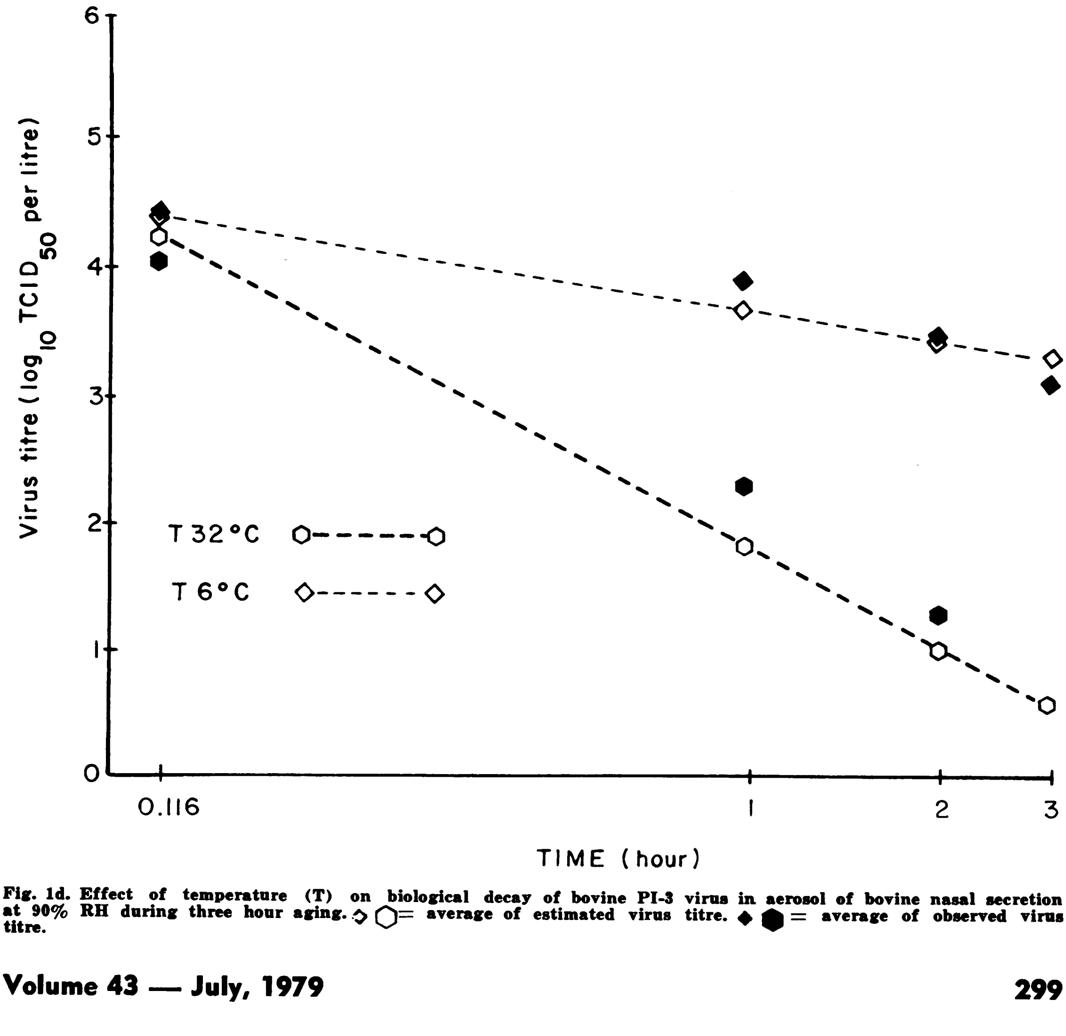

Fig. 1d. Effect of temperature (T) on biological decay of bovine PI-3 virus in aerosol of bovine nasal secretion at 90% RH during three hour aging. ◇ ○ = average of estimated virus titre. ◆ ⬢ = average of observed virus titre.

TABLE I. Recovery of Bovine Parainfluenza Type 3 Virus from Aerosols of Bovine Nasal Secretion from Noninfected Calf (BNIS) and Eagle's Minimum Essential Medium (EMEM) at Different Temperatures (T) and Relative Humidities (RH)

		Mean Virus Titre (Log_{10} $TCID_{50}$/Litre) ± SD[a]									
		EMEM					BNSI				
T	RH	0	7 min	1 h	2 h	3 h	0	7 min	1 h	2 h	3 h
32°C	30%	5.0 ±0.12	3.7 ±0.40	2.2 ±0.32	1.6 ±0.23	0.8 ±0.69	5.0 ±0.10	3.6 ±0.42	2.8 ±0.25	2.1 ±0.10	1.4 ±0.23
	90%	5.2 ±0.15	4.3 ±0.15	2.4 ±0.25	0.8 ±0.69	0.0 ±0.00	5.1 ±0.25	4.1 ±0.17	2.3 ±0.25	1.3 ±0.15	0.0 ±0.00
6°C	30%	5.4 ±0.23	4.1 ±0.25	3.8 ±0.26	3.7 ±0.35	3.2 ±0.67	5.1 ±0.10	4.1 ±0.17	3.8 ±0.12	3.5 ±0.17	2.6 ±0.51
	90%	5.2 ±0.31	4.8 ±0.38	4.2 ±0.21	4.1 ±0.15	3.8 ±0.10	4.9 ±0.26	4.4 ±0.32	3.9 ±0.23	3.5 ±0.21	3.1 ±0.47

[a]Standard deviation

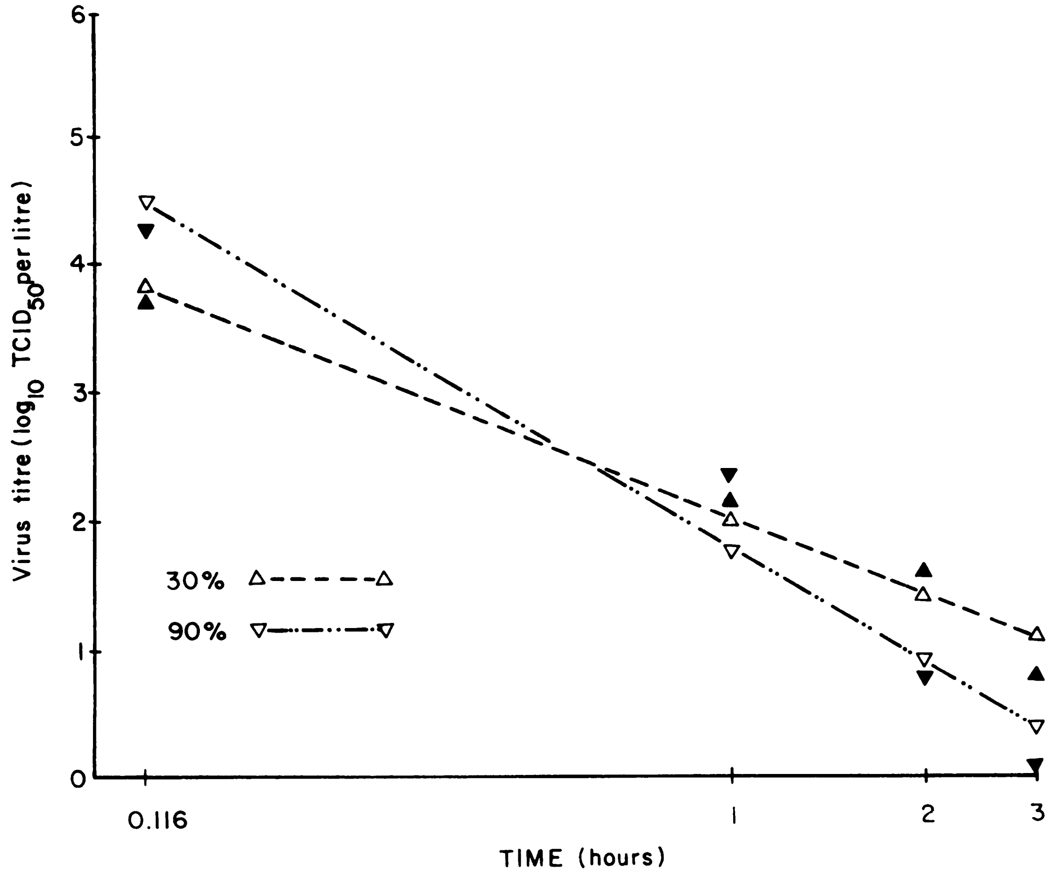

Fig. 2a. Effect of humidity (%) on biological decay of bovine PI-3 virus in aerosol of Eagle's minimum essential medium at 32°C during three hour aging. ▽△ = average of estimated virus titre. ▼▲ = average of observed virus titre.

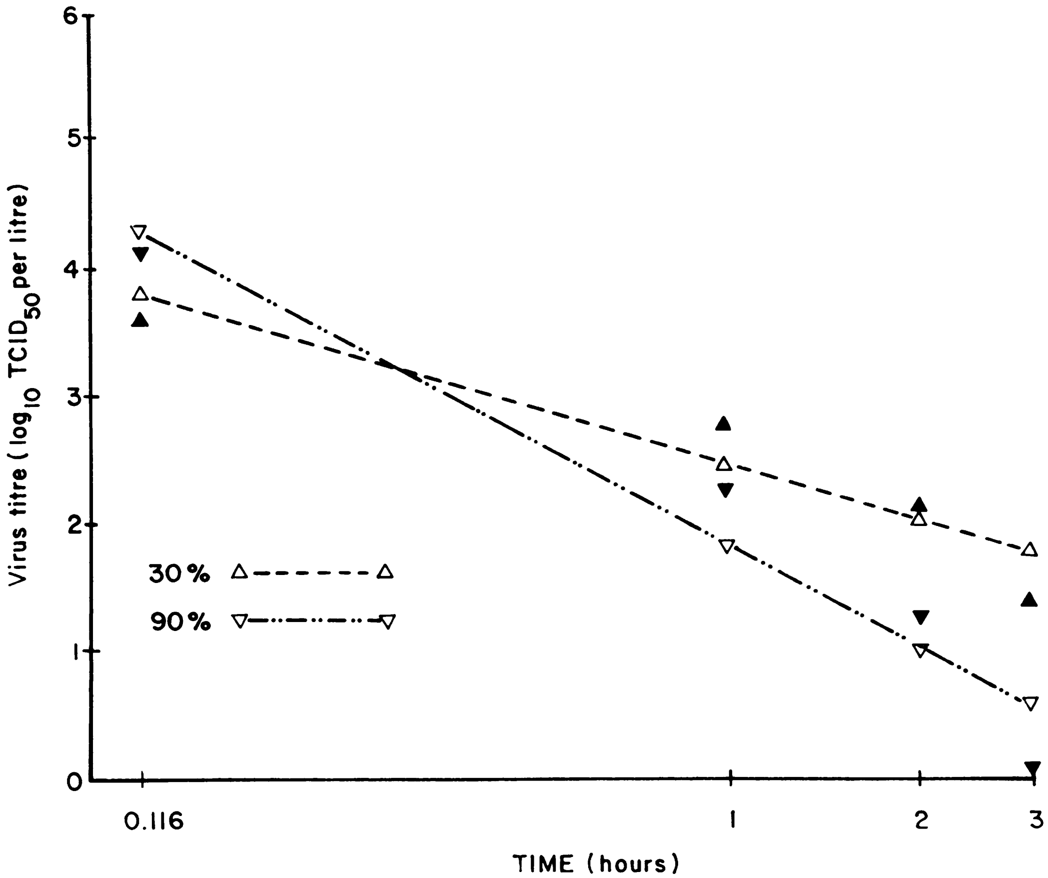

Fig. 2b. Effect of humidity (%) on biological decay of bovine PI-3 virus in aerosol of nasal secretion from noninfected calf at 32°C during three hour aging. ▽△ = average of estimated virus titre. ▼▲ = average of observed virus titre.

b) in aerosols of both media, while at 6°C the RH did not significantly influence the decay rate. Inactivation of the virus occurred more rapidly in an aerosol of EMEM than in an aerosol of NIBS only at 30% RH and 32°C (Fig. 3a) while the contrary was true at 6°C (Fig. 3b).

DISCUSSION

These data represent the first report on the stability of bovine PI-3 virus in aerosols of NIBS. The response of this virus in aerosols of bovine nasal secretion to different temperature and RH levels might give some indication of its behaviour under field conditions, while the observations on its stability in EMEM enable comparisons to be made with other viruses. During spraying, the type of media and the temperature had little influence on the virus decay rate and in general the decay rate did not seem to be greatly affected by RH. This seems to be in agreement with the observation of Miller and Artenstein (14) on human PI-3 virus in aerosols of Eagle's basal medium with 5% serum at room temperature, although Donaldson and Ferris (6) found that two bovine strains of PI-3 virus were sensitive to high humidity during aging for five minutes at room temperature.

At 6°C, the stability of PI-3 stored in aerosols did not significantly differ at either level of RH. At 32°C, however, 90% RH was markedly more harmful to the virus than 30%. This indicates that PI-3 exhibits a RH dependent stability pattern only at higher temperatures. Similarly, Ehrlich and Miller (7) reported that at

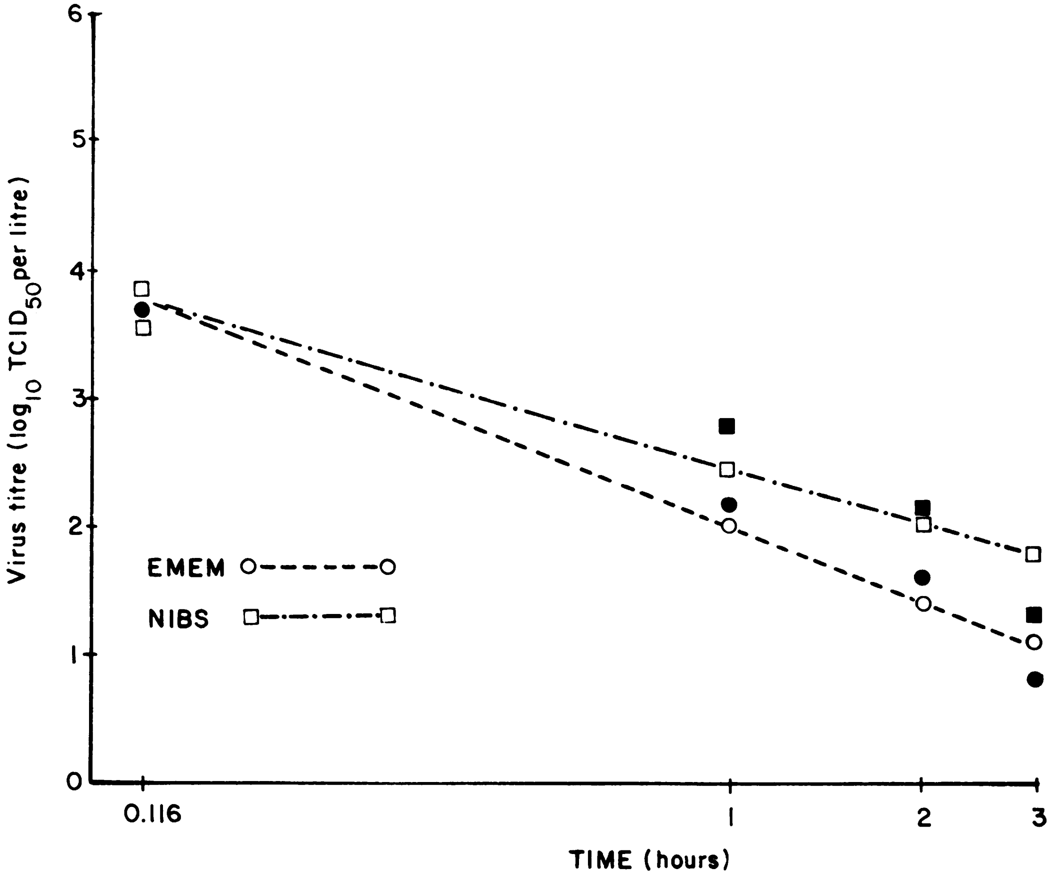

Fig. 3a. Effect of medium on biological decay of bovine PI-3 virus in aerosol at 32°C and 30% RH during three hour aging. ○ □ = average of estimated virus titre. ● ■ = average of observed virus titre. EMEM = Eagle's minimum essential medium. NIBS = nasal secretion from noninfected calf.

temperatures from −40°C to 24°C the decay rate of Venezuelan equine encephalitis virus aerosolized from a liquid suspension was independent of RH change between 18% and 90%. At 42°C, however, a tenfold increase in the biological decay rate was observed and higher RH values (70 to 90%) were most deleterious to the virus. Akers *et al* (1) observed that SV40 exhibited a RH-dependant (50%–60%) inactivation pattern only at high temperature (32°C). Hemmes (12), moreover, stated that human respiratory diseases occurring during the fall did not seem to be correlated with a particular level of RH.

In the present studies, in aerosols at 32°C the behaviour of bovine P1-3 seemed to resemble that of human PI-3 virus (14), Newcastle disease virus (18), measles virus (5) and human influenza virus (13) in aerosols held at room temperature in its response to RH, since high RH was associated with the higher biological decay rate.

The present findings also indicate that temperature had a major effect on virus decay rate in aerosols at both levels of RH and suggest that manipulation of the RH alone in an animal house is unlikely to control the spread of PI-3 virus. It is clear that at low temperature RH did not influence virus stability and it is probable that temperature exerts a more direct effect than RH on the virus decay rate. The fact that aerosols of PI-3 virus were more stable at the lower temperature may be related to the finding that field infections of cattle with PI-3 virus were more common in autumn and early winter in England (3).

The greater stability of the virus in BNS than in EMEM at 30% RH and 32°C and the reserve situation at 6°C might be attributed to differences in the composition of

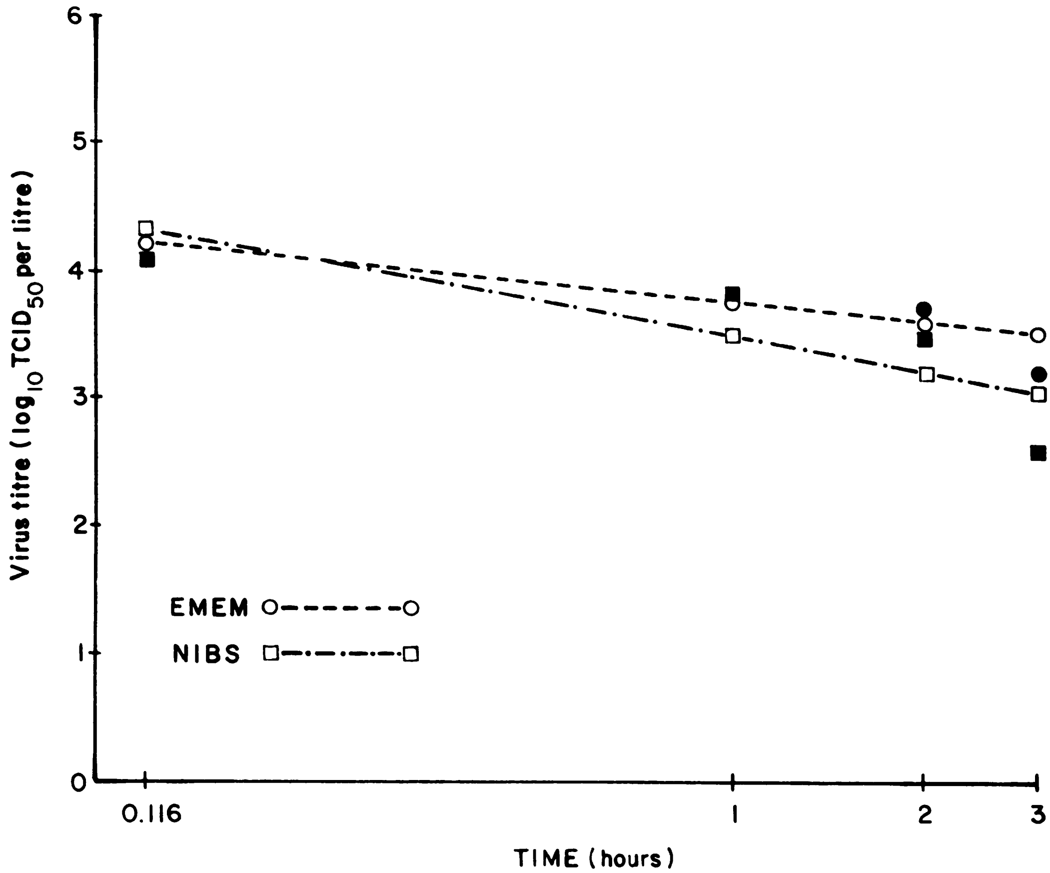

Fig. 3b. Effect of medium on biological decay of bovine PI-3 virus in aerosol at 6°C and 30% RH during three hour aging. ○ □ = average of estimated virus titre. ● ■ = average of observed virus titre. EMEM = Eagle's minimum essential medium. NIBS = nasal secretion from noninfected calf.

the two media which interact differently at particular RH and temperature levels. This observation stresses the importance of studying the virus in a natural medium such as nasal secretion to obtain information which might be of epidemiological value. Webb *et al* (22) demonstrated the tendency of simple or complex media to shift or reverse the virucidal action of certain levels of humidity while the protective action of certain media, especially at RH below 40% was shown by De Jong *et al* (4).

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